

**-pH suspected GHB/GBL sample as soon as possible to understand interconversion rates and which species of GHB could exist if asked in court.**

### **The Importance of pH**

#### **Forensic Issues of Interconversion**

- Deionized water at ambient temperature-GHB stable
  - ❖ GBL converts to GHB: 1% GHB after 1 day, < 3% after 16 days, 16% after 2 months and 33 % after 6-7 months
- pH 12- GHB stable and GBL rapidly converts to GHB
- pH 7- Conversion of GBL to GHB similar to base catalyzed mechanism
- pH 2- Start with GHB or GBL-mixture of both forms within 9 days

\*Source: L. Ciolino and M. Mesmer FDA Forensic Chemistry Center, February 2000 Academy Meeting Presentation

(\*Consideration for legal reclassification: Any Aqueous GBL solution has the potential to contain GHB)

### **To Screen for All Drugs**

-Filter or centrifuge samples that have an excessive amount of insoluble particulates.  
-Add 1 drop of liquid sample in a 1.8 mL vial and bring up to 1 mL with water. For very viscous samples, adjust volumes and dilute appropriately. For weak unknowns, samples may not need any diluting. If an unknown contains insoluble matter of forensic interest, consider adding 2 drops of chloroform. Perform a GC screen. Submit to GC/MS for screen testing using the GHBSCRN method. This will screen for GHB/GBL/1,4-BD and other controlled substances. Confirmatory work is described below.

### **To Confirm GBL in Aqueous Liquids**

-Filter or centrifuge samples that have an excessive amount of insoluble particulates.  
-Extract aqueous samples with an equal volume of CHCL3 to isolate GBL and to remove extraneous organic components. Isolate chloroform layer and wash 3-5X with water to remove GHB free acid. This should be validated for acidic solutions by drying chloroform and derivatizing residue.  
-Dry chloroform layer with anhydrous sodium sulfate.  
-Run the chloroform layer on the GC instrument with a 1mg/mL GBL standard prepared in chloroform. Use area counts to dilute unknowns to approximately 1 mg/mL GBL for GC/MS confirmation.  
-Chromatographic fronting/splitting exists for GBL in water on the GC/MS instrument. A chloroform GBL standard does not front/split and is better suited for confirmatory work. The chloroform layer will not extract GHB sodium salt or any other salt form of GHB

### **To Confirm 1,4-Butanediol (1,4-BD) in Aqueous Liquids**

-The NY DEA and New Hampshire State Police Forensic Laboratory have protocols that imply 1,4-butanediol can be extracted from aqueous liquids with chloroform. I was not able to validate this extraction procedure with any degree of success. However, the above procedure can be used to confirm 1,4-BD if chloroform is substituted with Petroleum Ether.

### **To Confirm GHB in Aqueous Solutions**

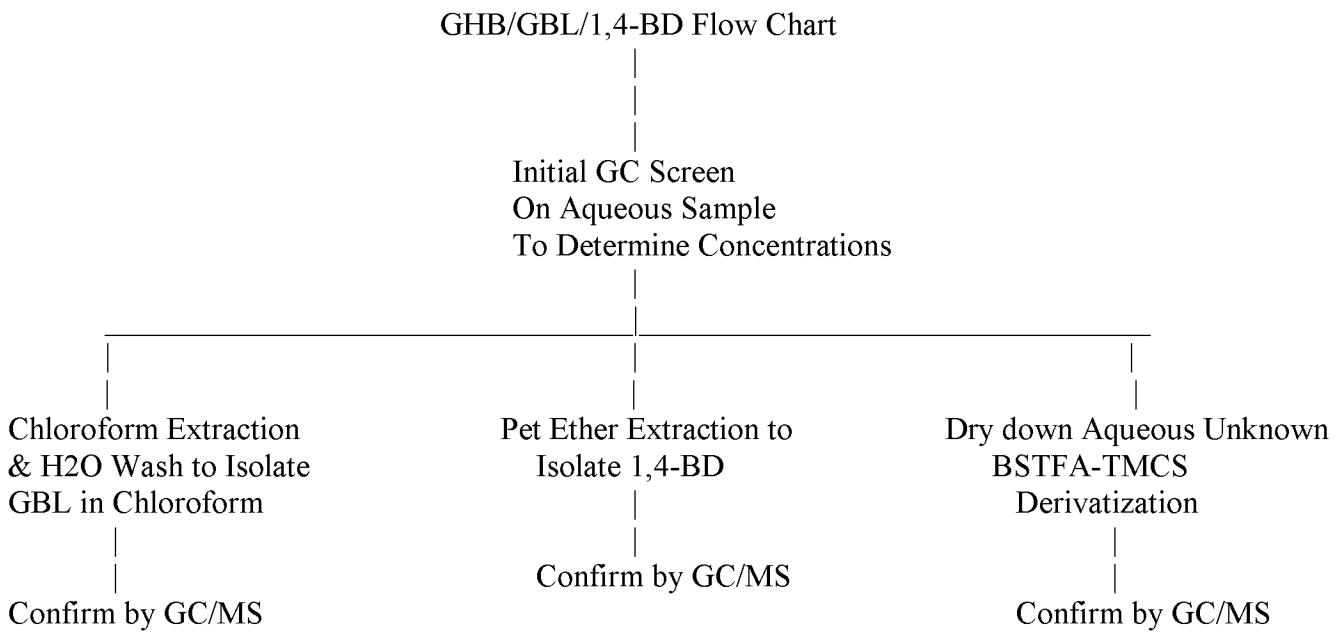
-If available, place 0.5 mL of diluted sample (from step #1) in a 1.8 mL vial (amber and calibrated) and extract with chloroform (optional). This step effectively removes GBL even though drying the sample will also remove GBL. Evaporate at room temperature using the Pierce Reacti-Therm unit. Two days may be required to thoroughly evaporate the water. Derivatize immediately after evaporating. This process will

remove GBL and 1,4-butanediol along with water by evaporation. Only GHB can be confirmed if present in the sample.

-While drying unknowns, remove 0.5 mL of a stock 1 mg/mL aqueous GHB standard solution for evaporation as well. GHB is stable in de-ionized water and any minor interconversion will be evaporated away.

-Once the sample (standard) is dried down, add 200ul of BSTFA-TMCS. Let sit at room temperature for 2-3 hours, mixing/vortex repeatedly. "BSTFA-TMCS has good solvent properties and can function as a silylation reagent without additional solvents." \*Supelco Certificate of Analysis

-After derivatization, add an appropriate volume of acetonitrile to bring the final volume back to 0.5 mL. Confirm using GC/MS with a derivatized GHB standard within 24-48 hours. Submit a blank consisting of BSTFA-TMCS and acetonitrile to rule out GHB contamination of the solvents (injected once for QC purposes) and also a straight acetonitrile blank to be used in between samples and standards.



#### Reference

\* The Extraction and Infrared Identification of Gamma Hydroxybutyric Acid (GHB) from Aqueous Solutions